

New terpenoids from the roots of *Jatropha curcas*YANG YuanFeng<sup>1,2</sup>, LIU JieQing<sup>1</sup>, LI XuYang<sup>1,2</sup>, LIU EnQian<sup>1</sup>, LI ZhongRong<sup>1</sup> & QIU MingHua<sup>1,2\*</sup><sup>1</sup> State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China;<sup>2</sup> University of Chinese Academy of Sciences, Beijing 100049, China

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Two new sesquiterpenoids, (1*S*,2*R*)-dihydroxycycloax-4(15)-ene (**1**), 14-dehydroxyl daucucarotol (**2**), and one new rhamnofalane diterpenoid, 2-hydroxy-3-dehydroxycanjoane (**3**), together with two known compounds, curcusone D (**4**) and curcusone C (**5**), were isolated from the roots of *Jatropha curcas*. The chemical structures of these compounds were established by chemical methods and extensive 1D- and 2D-NMR spectroscopic data analyses.

*Jatropha curcas*, sesquiterpenoids, diterpenoid

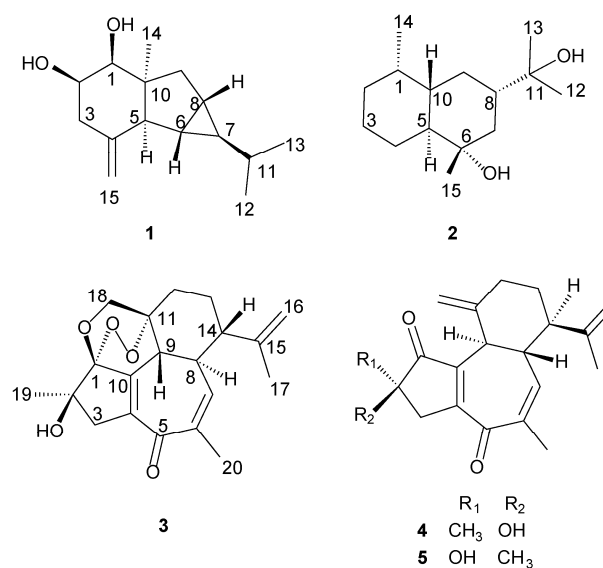
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*Jatropha curcas* (Euphorbiaceae) is indigenous to southwest and southeast China. The plant has been used as folk medicine for the treatment of injuries and swelling, fracture, skin itching, eczema, and acute gastroenteritis embolism [1]. Besides, the seeds containing 30% oil can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine. And that is why *Jatropha curcas* was greatly focused as biodiesel source all over the world. The plants of genus *Jatropha*, mainly distributed in tropical and subtropical America [2]. The chemical constituents from this genus were plentiful, covered of diterpenoids, sesquiterpenoids, lignans and coumarins, flavonoids, and some others [3], and some of the diterpenoids have been proved to be effective as anti-inflammatory [4], antiproliferation [5,6], antiplasmodial [7], antituberculosis [8], antitumor [8,9], and cytotoxic activities [10].

Lately, several structurally interesting diterpenoids were isolated from this plant [9,11,12]. Its potential medicinal value and the diverse constituents prompted us to initiate the chemical investigation of *J. curcas*.

Our phytochemical study from the methanol extracts of roots of *J. curcas* led to the isolation of five compounds

(**1–5**) (Figure 1), including two new sesquiterpenes, (1*S*,2*R*)-dihydroxycycloax-4(15)-ene (**1**) and 14-dehydroxyl daucucarotol (**2**), one new rhamnofalane endoperoxide, 2-hydroxy-3-dehydroxycanjoane (**3**), and two known



**Figure 1** Structures of compounds **1–5**.

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compounds, curcusone D (**4**) [13] and curcusone C (**5**) [13]. This paper deals with the isolation and structure elucidation of new compounds **1**–**3**.

## 1 Experimental

### 1.1 General experimental procedures

UV spectra were obtained in MeOH with a Shimadzu double-beam 22210A spectrophotometer; Optical rotations were measured on a SEPA-300 polarimeter; IR spectra were obtained using a Bio-Rad FTS-135 infrared spectrometer with KBr pellets; NMR spectra were recorded on Bruker DRX-600 (600/150 MHz) spectrometers with TMS as internal standard; high resolution electrospray ionization mass spectroscopy (HRESIMS) and low resolution ESIMS were recorded on an APIQSTAR time-of-flight mass spectrometer while high resolution electron impact mass spectroscopy (HREIMS) were measured on a VG Auto Spec-3000 mass spectrometer; Semipreparative HPLC was performed on an Agilent 1100 apparatus equipped with a diode-array detector and a YMC-PackProC<sub>18</sub>RS (YMC, 250 mm × 10 mm, 5 μm) column; column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), Sephadex LH-20 (General Electric Company, Fairfield, CT); Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H<sub>2</sub>SO<sub>4</sub>.

### 1.2 Plant material

The roots of *Jatropha curcas* were collected from E-Shan county, Yunnan Province, China, in July 2010, and identified by Prof. Hua Peng. A voucher specimen (No. KIB 20100701) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

### 1.3 Extraction and isolation

The dried and powdered roots of *J. curcas* (5 kg) were refluxed three times with 95% aqueous MeOH at room temperature. The combined MeOH extracts were concentrated under reduced pressure, and then partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The CHCl<sub>3</sub> layer (130 g) was subjected to a macroporous resin gel column chromatography eluted with a gradient solvent system of MeOH–H<sub>2</sub>O to yield 80% MeOH–H<sub>2</sub>O extract. The 80% MeOH–H<sub>2</sub>O extract (70 g) was chromatographed on silica gel CC with Me<sub>2</sub>CO–PE mixtures (1:15, 1:10, 1:5, and 1:1) to get four fractions (Fr.1, Fr.2, Fr.3, and Fr.4). Fr.3 (10 g) was separated on an ODS column eluting with MeOH–H<sub>2</sub>O (45%, 60%, and 70%) to give subfractions Fr.3.1–Fr.3.3. Subfraction Fr.3.1 (3 g) was further isolated and purified by silica gel column (PE–

Me<sub>2</sub>CO, 10:1), Sephadex LH-20 (MeOH), Preparative TLC (CH<sub>3</sub>Cl–Me<sub>2</sub>CO) to yield **1** (10 mg) and **2** (7 mg). Fr.1 (20 g) was further fractionized on an ODS column eluting with MeOH–H<sub>2</sub>O (55%, 65%, and 80%) to afford subfractions Fr.1.1–Fr.1.3. Subfraction Fr.1.3 (5 g) was further isolated and purified by silica gel column (PE–Me<sub>2</sub>CO), semipreparative HPLC (CH<sub>3</sub>CN–H<sub>2</sub>O) to afford **3** (1.3 mg). Fr.2 (20 g) was further fractionized on an ODS column eluting with MeOH–H<sub>2</sub>O (50%, 62%, and 75%) to afford subfractions Fr.2.1–Fr.2.3. Subfraction Fr.2.2 (8 g) was further isolated and purified by silica gel column (PE–Me<sub>2</sub>CO) to afford **4** (40 mg) and **5** (30 mg).

(i) (1*S*,2*R*)-Dihydroxycycloax-4(15)-ene (**1**). Yellow, oil;  $[\alpha]_D^{25} = -33.2$  (*c* 1.0, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$ : 3350, 2958, 2953, 2870, 1047 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; positive ESIMS (pos.): *m/z* 259 [M + Na]<sup>+</sup>; positive HRESIMS (pos.): *m/z* 259.1668 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>24</sub>O<sub>2</sub> [M + Na]<sup>+</sup>, *m/z* 259.1673).

(ii) 14-Dehydroxyl daucucarotol (**2**). White, amorphous powder;  $[\alpha]_D^{25} = +2.8$  (*c* 1.0, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$ : 3340, 2950, 2933, 2865, 1056 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; positive ESIMS (pos.): *m/z* 263 [M + Na]<sup>+</sup>; positive HRESIMS (pos.): *m/z* 263.1985 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>28</sub>O<sub>2</sub> [M + Na]<sup>+</sup>, *m/z* 263.1987).

(iii) 2-Hydroxy-3-dehydroxycanjoane (**3**). White, crystalline solid;  $[\alpha]_D^{25} = -63.2$  (*c* 0.03, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 254 (3.00), 202 (5.19) nm; IR (KBr)  $\nu_{\max}$ : 3440, 1711, 1628, 1452 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data see Table 1; positive TOF MS *m/z* 345 [M + H]<sup>+</sup>; positive HREIMS *m/z* 367.1742 [M + Na]<sup>+</sup> (calcd for C<sub>20</sub>H<sub>24</sub>O<sub>5</sub> [M + Na]<sup>+</sup>, *m/z* 367.1521).

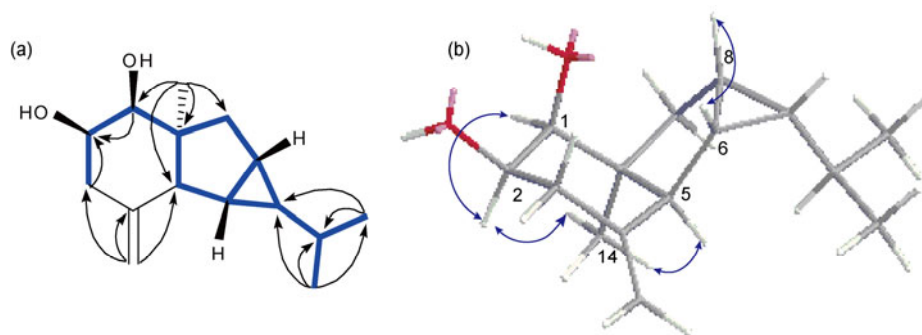
## 2 Results and discussion

Compound **1** was obtained as yellowish oil. Its molecular formula was determined as C<sub>15</sub>H<sub>24</sub>O<sub>2</sub> by positive HRESIMS *m/z* 259.1668 [M + Na]<sup>+</sup> (calcd for C<sub>15</sub>H<sub>24</sub>O<sub>2</sub> [M + Na]<sup>+</sup>, *m/z* 259.1673). The IR spectrum exhibited absorption bands for OH group (3350 cm<sup>-1</sup>) and an olefinic function (1047 cm<sup>-1</sup>). The <sup>1</sup>H, <sup>13</sup>C NMR, and DEPT (Distortionless enhancement by polarization transfer) data of **1** (Table 1) showed the presence of three methyls (two geminal methyls), three methylenes (one olefinic), seven methines (two oxygenated), and two quaternary carbons (one olefinic). The above-mentioned data, together with the evidence of four degrees of unsaturation suggested that **1** was quite similar to (1*R*,2*R*)-dihydroxycycloax-4(15)-ene [14].

Comprehensive analysis of HSQC (heteronuclear single quantum correlation) and <sup>1</sup>H–<sup>1</sup>H COSY (correlation spectroscopy) spectra of **1** allowed the establishment of one structural fragment as drawn in blue bold lines in Figure 2(a). One exocyclic double bond was evident from <sup>1</sup>H NMR signal at  $\delta_H$  4.98 and <sup>13</sup>C NMR signals at  $\delta_C$  106.5 (t). In the

**Table 1**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1–3** in  $\text{CDCl}_3$  (600 and 150 MHz, respectively)

	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, $J$ in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (mult, $J$ in Hz)
1	82.4 (d)	3.72 (d, 8.5)	39.5 (d)	2.02–2.05 (m)	103.8 (s)	
2	72.7 (d)	4.12 (ddd, 10.6, 8.8, 6.5)	34.6 (t)	1.60–1.64 (m) 1.29–1.32 (m)	77.6 (s)	
3	44.4 (t)	2.88 (dd, 13.2, 7.5) 2.43 (brdd, 13.2, 5.9)	27.3 (t)	1.96–1.99 (m)	43.9 (t)	2.80–2.84 (m) 2.63 (dd, 16.1, 4.1)
4	145.8 (s)		24.1 (t)	2.22 (ddd, 22.6, 15.1, 7.6) 1.27–1.30 (m)	138.9 (s)	
5	58.6 (d)	1.88 (d, 3.5)	53.0 (d)	2.20 (m)	193.6 (s)	
6	32.7 (d)	0.85–0.94 (m)	74.3 (s)		137.1 (s)	
7	48.4 (d)	0.50–0.55 (m)	48.9 (t)	1.88 (m) 2.24 (m)	138.6 (d)	5.94 (dd, 4.0, 1.5)
8	25.5 (d)	1.18–1.24 (m)	51.1 (d)	1.93 (m)	35.3 (d)	2.87 (d, 10.8)
9	43.5 (t)	2.24 (dd, 12.1, 6.6) 1.25–1.27 (m)	30.2 (t)	1.70–1.73 (m) 1.54–1.58 (m)	47.2 (d)	2.45–2.49 (m)
10	59.5 (s)		41.0 (d)	1.81–1.84 (m)	147.3 (s)	
11	24.2 (d)	1.32–1.37 (m)	73.4 (q)		69.8 (s)	
12	22.2 (q)	0.90 (d, 4.5)	28.7(q)	1.37 (s)	32.2 (t)	1.53–1.55 (overlapped) 1.36 (m)
13	22.0 (q)	0.93 (d, 4.5)	26.4(q)	1.32 (s)	25.7 (t)	1.88 (ddd, 9.1, 4.5, 2.5) 1.53–1.55 (overlapped)
14	15.9 (q)	1.18 (s)	15.9 (q)	0.85 (d, 7.0)	51.3 (d)	2.01–2.05 (m)
15	106.5 (t)	4.98 (dd, 3.7, 1.4 )	22.4 (q)	1.34 (s)	146.5 (s)	
16					113.3 (t)	4.80, 4.77 (s)
17					18.9 (q)	1.60 (s)
18					70.0 (t)	4.01–4.04 (m) 3.41–3.43 (m)
19					23.6 (q)	1.39 (s)
20					20.5 (q)	1.76 (s)

**Figure 2** The  $^1\text{H}$ - $^1\text{H}$  COSY (—), key HMBC correlations (H→C) (a), and key ROESY correlations (↺↻) (b) of **1**.

HMBC spectrum of **1**, the correlations from the olefinic proton signal  $\delta_{\text{H}}$  4.98 to C-3, C-4, and C-5 indicated the assignment of the exocyclic double bond between C-4 and C-15. The HMBC (heteronuclear multiple bond correlation) correlations from the quaternary methyl signal at  $\delta_{\text{H}}$  1.18 to C-1, C-5, C-9, and C-10, and from one of the methylene proton signals at  $\delta_{\text{H}}$  2.88 (dd, 13.2, 5.9) to C-1 and C-2, suggested the presence of hydroxyl groups at C-1 and C-2,

respectively. Moreover, the existence of strong NOE correlations between H-1/H-2, H-2/H<sub>3</sub>-14, H<sub>3</sub>-14/H-5, and H-6/H-8 in the ROESY (rotating frame overhauser effect spectroscopy) spectrum of **1** (Figure 2(b)) suggested that OH-1 was  $\beta$ -oriented, which was the only difference between **1** and (1*R*,2*R*)-dihydroxycycloax-4(15)-ene. Consequently, the structure of **1** was deduced as (1*S*,2*R*)-dihydroxycycloax-4(15)-ene.

Compound **2** obtained as white powder, possessed a molecular formula of  $C_{15}H_{28}O_2$ , as evidenced by HRESIMS at  $m/z$  263.1985  $[M + Na]^+$  (calcd for  $C_{15}H_{28}O_2$   $[M + Na]^+$ ,  $m/z$  263.1987), in accordance with two degrees of unsaturation. Importantly, the absence of any olefinic moieties in the  $^{13}C$  NMR spectrum required the presence of two rings to satisfy the degrees of unsaturation.

The  $^{13}C$  NMR and DEPT (Table 1) data of **2** exhibited four methyls, five methylenes, four methines, and two oxygenated quaternary carbons. These spectral features were closely related to those of daucucarotol [15], except for a tertiary methyl signal in **2** instead of an oxygenated methylene signal in daucucarotol. The HMBC spectrum of **2** displayed cross-peaks from the doublet methyl signal at  $\delta_H$  0.85 to C-1, C-2, and C-10, which suggested that the doublet methyl group located at C-1. The planar structure of **2** was further established from the  $^1H$ - $^1H$  COSY and HMBC spectra (Figure 3(a)).

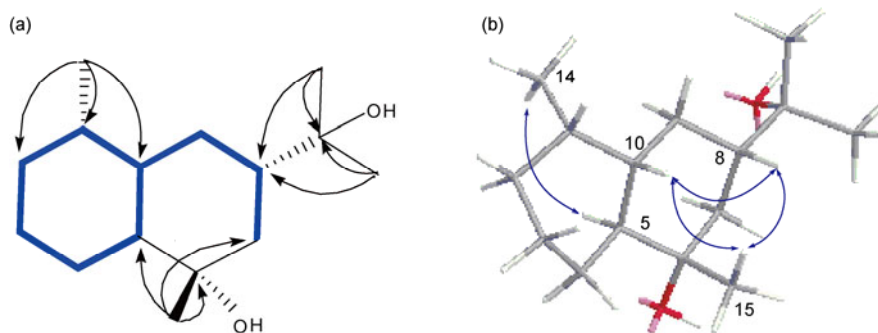
The ROESY (Figure 3(b)) correlations of Me-14/H-5, H-10/H-8, and H-10/Me-15 suggested that the H-1, H-8, Me-15, and H-11 were of  $\beta$ -orientation. Ultimately, the structure of **2** was determined and named 14-dehydroxyl daucucarotol.

Compound **3** was obtained as crystalline solid. The HREIMS spectrum gave an  $[M + Na]^+$  ion at  $m/z$  367.1742, corresponding to the molecular formula  $C_{20}H_{24}O_5$ . The IR spectrum displayed absorption bands for unsaturated carbonyl ( $1658\text{ cm}^{-1}$ ) and olefinic ( $1628\text{ cm}^{-1}$ ) groups. The  $^1H$ ,  $^{13}C$ , and DEPT (Table 1) NMR data of **3** showed the pres-

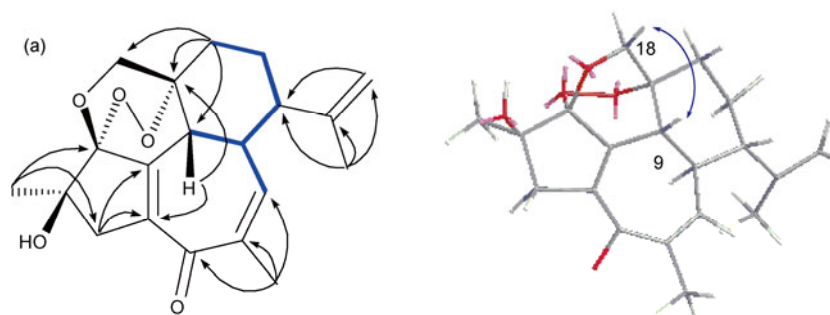
ence of three methyls, one oxymethylene ( $\delta_C$  70.0), one dioxygenated carbon ( $\delta_C$  103.8), two oxygenated carbon, a terminal double-bond, two double-bonds, and a keto carbonyl carbon ( $\delta_C$  193.6). These data showed that **3** was similar with caniojane [16]. The key difference was that one oxygenated quaternary carbon signal in **3** instead of an oxygenated methine in caniojane. Meanwhile, there was no doublet methyl signal in  $^1H$  NMR spectrum. Above data indicated that a hydroxyl group would attach to C-2, which was confirmed by the HMBC correlations from H<sub>3</sub>-19 to C-1, C-2 ( $\delta_C$  77.6, s), and C-3.

The planar structure of **3** was further determined by the correlations in the HMBC and  $^1H$ - $^1H$  COSY spectra (Figure 4(a)). The relative configuration of Me-19 was determined as  $\alpha$ -oriented on the basis of the chemical shift of C-19 at  $\delta_C$  23.6 and were consistent with those reported for curcusone C [13]. The relative configuration of the peroxy bond between C-1 and C-11 was assigned as  $\beta$  by the correlations between H<sub>2</sub>-18 and H-9 in the ROESY (Figure 4(b)) spectrum. Finally the structure of **3** was proposed to be 2-hydroxy-3-dehydroxycaniojane.

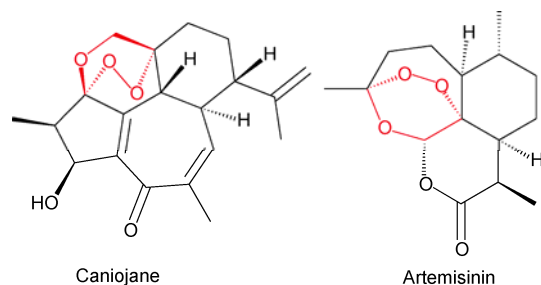
Caniojane and 1,11-bisepicaniojane [16,17], having peroxide bridge, performed certain antimalarial activity [18]. Well-known to all, artemisinin (Figure 5) is the antimalarial drug containing peroxide bridge, and the peroxy bond is necessary for the activity based on the structure-activity relationships. So, whether compound **3** with peroxide bridge playing a similar role in the resistance of plasmodium activity needs further study.



**Figure 3** The  $^1H$ - $^1H$  COSY (—), key HMBC correlations (H→C) (a), and key ROESY correlations (↪) (b) of **2**.



**Figure 4** The  $^1H$ - $^1H$  COSY (—), key HMBC correlations (H→C) (a), and key ROESY correlations (↪) (b) of **3**.



**Figure 5** Structures of caniojane and artemisinin.

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